TECHNICAL ABSTRACT

Background: It has been shown that terminal end buds, which are the prospective site of stem cells during ductal morphogenesis, are the targets of carcinogen-induced transformation in rat breast cancer models. It has also been established that stem cell markers are upregulated in human breast cancer. Therefore, it seems that stem cells play an important role in breast cancer onset and development. To understand the biology of stem cells and their role in malignant transformation it is essential to determine 1) the stem cell organization *in situ*, 2) the stem cell niche -the physical microenvironment and molecular milieu within which the stem cell resides- and 3) the conditions that allow for stem cell expansion and differentiation. Bromodeoxyuridine (BrdU) label retention has extensively been used to identify stem-cell-containing populations: label-retaining cells (LRCs) have the general properties anticipated for epithelial stem cells, including a slow cell cycle, occupying a fixed position in relation to tissue architecture, and behaving like clonogenic cells *in vitro*.

Objective/Hypothesis: Several lines of evidence indicate that the dorsal skin of mice is organized into hexagonal domains called epidermal proliferating units (EPUs). Each of these structures has a central cell that replicates infrequently and is presumed to be a stem cell. Thus, EPUs are considered stem cell compartments. Surrounding the central cell there are more rapidly proliferating cells that give rise to vertically migrating differentiating keratinocytes. In murine intestinal crypts, stem cells are arranged in an annulus at the fourth cell position from the crypt base. The daughters of these stem cells undergo a limited and defined number of divisions. These daughter cells are found in the midcrypt region and can mature into one of five different cell types. As they mature, cells differentiate so that cells of the upper crypt cannot regenerate the crypt after radiation injury.

These two examples suggest that epithelial stem cells are organized in columnar structures or functional units, with the stem cells in one end of the structure and the completely differentiated cells in the other end. Because epithelial stem cells give raise to these architectural domains in both mouse epidermis and murine intestinal crypts, we hypothesize that they give raise to similar structures in the mouse mammary gland.

Specific Aims: (1) Determine the location of the label retaining cells in the normal mouse mammary gland, (2) establish the three-dimensional distribution of these cells and compare it to the ones seen in mouse epidermis and murine intestinal crypts, and (3) develop new three-dimensional image analysis tools in order to be able to achieve aims (1) and (2). We also want to (4) repeat (1) and (2) in transgenic mice overexpressing the *c-neu* gene. These mice develop focal tumors with long latencies (in the order of months), and that allows for the study of cancer progression from hyperplastic stages to invasive ones. Thus, we will investigate the changes of the LRCs and their niche in tumor onset and development, and their contribution to these events

Study Design: BrdU label retention will be used to identify the location of stem cells in the mouse mammary gland. The glands will be sliced into thin sections. Immunofluorescence will be used to stain the LRCs. The sections will be imaged and these images will be used to create three-dimensional reconstructions of the glands. The LRCs will be segmented in the images and their three-dimensional distribution will be studied using 3D spatial pattern analysis. The results of these studies will be validated at the confocal microscope and compared to those observed in other organs (e.g.: epidermis, intestinal crypts). The experiments will be repeated using transgenic mice overexpressing the *c-neu* gene.

Relevance: Modulation of stem cell behavior holds exceptional promise for a new prophylactic approach for controlling cancer risk in general and breast cancer risk in particular. The three-dimensional characterization of the distribution of stem cells and their niche *in situ* will be an important step towards understanding stem cell behavior and, therefore, towards new modalities of cancer prevention and therapy.